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PBB Fed to Adult Female Chickens: Its Effect on Egg Production, Reproduction, Viability of Offspring, and Residues in Tissues and Eggs

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Two experiments were conducted with light breed laying chickens fed polybrominated biphenyl (PBB) as FireMaster FF-1. The first involved feeding PBB at dietary levels of 0.2, 1, 5, 25, 125, 625, and 3125 ppm, the second involved levels of 30, 45, 60, 90, and 120. Each group had 24 hens, and each experiment had a control group of 24 hens. PBB diets were fed for 5 weeks. Feed intake, production, reproduction, tissue residues and viability of offspring were monitored during that time and a subsequent 8 weeks.

Production, hatchability, and viability of offspring were significantly affected by feeding PBB at 45 ppm. Marked inanition occurred at levels of 625 and 3125 ppm, and there was some loss of feed intake at 125 ppm. There was a return to normal production and hatchability in 3 to 4 weeks after PBB withdrawal of diets with levels of 125 ppm or less. Dose–response lines are presented for PBB in muscle, liver, kidney, adipose tissue, and eggs. Withdrawal curves for PBB from these tissues are also given.

Introduction

Two laboratories have been involved with elucidating the toxic effects of polybrominated biphenyls (PBB) in chickens since the 1973 accidental contamination of PBB in feeds within Michigan. Two preliminary reports appeared from the USDA, Agricultural Research Service in 1973 on the consequences of 20 ppm PBB given to laying hens (*1*, *2*). This was pure serendipity; the cause of the problem in Michigan was not yet known. However, the experience gained with the PBB compound was no doubt responsible for the eventual establishment by Dr. Fries of the USDA that the problem was due to PBB contamination. In the spring of 1974, extensive studies on PBB contamination were initiated at Michigan State University with the first report given in August, 1975 (*3*). That same month, Cecil and Bitman (*4*) from the USDA reported on extensive studies they had completed. There were some slight differences in the data from the two laboratories on the minimum level in the diet for an adverse effect on reproduction, the most sensitive criterion of several evaluated. The data from Michigan State University were developed on levels ranging from 0.2 to 3125 ppm. In these studies no adverse effect on hatchability or chick viability was detected at 25 ppm, in contrast to the studies (*1*, *2*) showing a PBB effect at 20 ppm. The details of our data on this study, and a subsequent one involving PBB levels of 30 to 120 ppm are reported in this paper. Some of the data appeared in an earlier report (*5*) in which PBB and PCB were compared for their toxicity in the chicken and quail. That report included data which had appeared from the USDA laboratory (*4*, *6*) providing details on the results reported in 1973 and 1975.

There is a very important difference in the type of PBB used by the two laboratories in their investigations. The compound used at Michigan State University was a sample from a bag representative of the material involved in the contamination. This material is FireMaster FF-1, the product of Michigan Chemical Corporation, which contains added anticaking compounds. The compound used at the USDA laboratories, known as FireMaster PB-6, was the product of the company which lacks the

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small (unknown) amounts of the anticaking compounds, and which had not been milled to obtain a free-flowing compound. Thus, particle size may be another difference between the two compounds under evaluation.

Methods and Procedures

The first experiment was started in June, 1974: the second in January, 1975. Both were conducted at the Michigan State University Poultry Research and Teaching Center. An end room of one of the buildings was used so that the experiments could be isolated from other studies in the building. The adjacent room was kept vacant. Eventually, all wastes, left-over feed, eggs, and chickens in the experiment were buried in a specially selected site to prevent contamination of ground water. The White Leghorn laying chickens in experiment 1 were 10 months in production (about 60 weeks of age) at the time they were assigned at random to one of seven treatments or to a control group. Each group consisted of 24 hens. The hens were in colony cages, each holding six hens. Feed and water were supplied *ad libitum*. Treatment levels, as shown in Table 1, were selected to start at 0.2 ppm with levels increasing at fivefold intervals. PBB diets were fed for 5 weeks, at which time three hens from each group were selected at random and sacrificed to obtain tissue samples of adipose, liver, and muscle. Then, during the last day of weeks 2, 4, 6, and 8 following withdrawal of PBB diets, three hens from each group were sacrificed for tissues. On the Thursday of every week of the experiment, eggs were saved for measurements of weights of yolk, albumen and shell, as well as examination for any unusual physical appearance. Eggs from this collection were set aside and frozen for eventual PBB analysis by the State of Michigan, Department of Agriculture Laboratory. All other eggs collected during the week were saved for incubation. Adult male chickens, kept in a separate room, were fed clean feed *ad libitum* and used to supply semen for artificial insemination of the hens.

Experiment 2 was initiated on January 23, 1975. White Leghorn hens 36 weeks of age were used. Again, 24 hens were randomly assigned to each of five treatments with PBB at 30, 45, 60, 90, and 120 ppm, and 24 hens to a control group. PBB diets were fed for 5 weeks and then withdrawn to follow the resultant effect on reproduction during the next 8 weeks.

In addition to measurements on reproduction, which included egg production, fertility of eggs, and embryo mortality during hatch, feed consumption, and mortality of offspring were also monitored. The latter was ascertained by rearing the hatched chicks from treated hens for 21 days on a control chick starter ration.

Tissue and egg samples submitted to the laboratory for the Michigan Department of Agriculture for assay of PBB were coded. After gas chromatographic analyses these data were returned to us for collating and data processing. PBB analysis was performed on a homogenized sample weighed accurately to 4.0 to 5.0 g in a tared aluminum moisture dish. The sample was covered with granular anhydrous Na_2SO_4 and mixed in with a small stirring rod. The sample was then dried at 100°C for 30 min and extracted on a Soxhlet apparatus into a tared flask with 6% ethyl ether in petroleum ether for 4–8 hr. The extract was brought to dryness, the flask weighed and the percent fat calculated. Following this, the fat was quantitatively transferred to

Table 1. Feed intake of laying chickens in experiments 1 and 2.[a]

Experiment 1[b]			Experiment 2		
PBB in diet, ppm	No. of hens	Feed intake, g/bird/day[c]	PBB in diet, ppm	No. of hens	Feed intake, g/bird/day[c]
None (controls)	24	99.1 (± 2.94)	None	24	117.6 (± 5.0)
0.2	24	98.8 (± 3.07)	30	24	113.8 (± 5.2)
1.0	24	106.1 (± 5.20)	45	24	113.4 (± 13.8)
5	24	99.6 (± 3.87)	60	24	113.4 (± 3.9)
25	24	99.4 (± 3.62)	90	24	116.4 (± 12.7)
125	24	93.9 (± 3.12)[d]	120	24	119.2 (± 8.1)
625	24	28.4 (± 4.00)[e]			
3125[f]	24	10.1 (± 11.7)[e]			

[a] PBB was fed for 5 weeks at graded levels of 0.2 to 3125 ppm (mg/kg diet) and 30 to 120 ppm.
[b] From data of Ringer and Polin (*5*).
[c] Mean (± std. dev.).
[d] $p < 0.05$.
[e] $p < 0.01$.
[f] Four weeks on PBB diet.

an approximately 40 g of activated Fluorisil column topped with anhydrous sodium sulfate. The sample was then eluted with 200 ml petroleum ether–ether (94:6). The petroleum ether as evaporated on a steam bath, and the residue from the column chromatography was resolved with high-temperature GLC by either one or both of the following procedures after diluting the residue to 5 ml with hexane. One procedure used a ^{3}H-foil electronic detector with a temperature of 220°C in the column and detector, and 250°C in the injector. The other used a ^{63}Ni-detector at a temperature of 270°C in the column and 310°C in the detector and 300°C in the injectorport. Nitrogen was the carrier gas in both procedures at a flow rate of 100 ml/min. The column was 182.9 × 0.64 cm in both procedures and was packed with 3% OV-1 on Gaschrome Q, 60-80 mesh. The normal working range for PBB analyses was 25–400 pg. Average recovery of spiked samples was 97.8%.

Results

Feed Intake

In the first experiment, adult female chickens showed a small but significant ($p < 0.05$) decline in food intake when fed a diet containing 125 ppm PBB (Table 1). Hens fed diets with PBB levels lower than this consumed normal amounts of feed. The decline in feed intake was drastic for those hens offered the diet with 3125 ppm PBB. These hens were starving themselves to death, so at the end of the fourth week, a week earlier than the schedule had indicated, these hens were switched to clean diets. Cecil and Bitman (*4*) reported that pair-fed controls in their experiment as well as hens fed PBB at 640 and 2000 ppm in the diet died within 30 days with the effect of PBB producing death because of starvation. Babish et al. (*7*) noted that Japanese quail refused to eat a semipurified diet containing 500 ppm PBB, but showed no inanition at 10, 20, or 100 ppm.

Egg Production

Coincident with the dramatic reduction in feed intake by laying hens fed 625 or 3125 ppm PBB in their diets was the complete loss of egg production within two weeks (Fig. 1). Interpretation of these data without pair-fed controls to separate out that effect on egg production caused by PBB vs. that produced by loss of nutrient intake, was not possible. However, investigators (*8*, *9*) established that hens cease egg production when without feed for longer than two days. Egg production in the group of hens provided with 125 ppm PBB in their diet

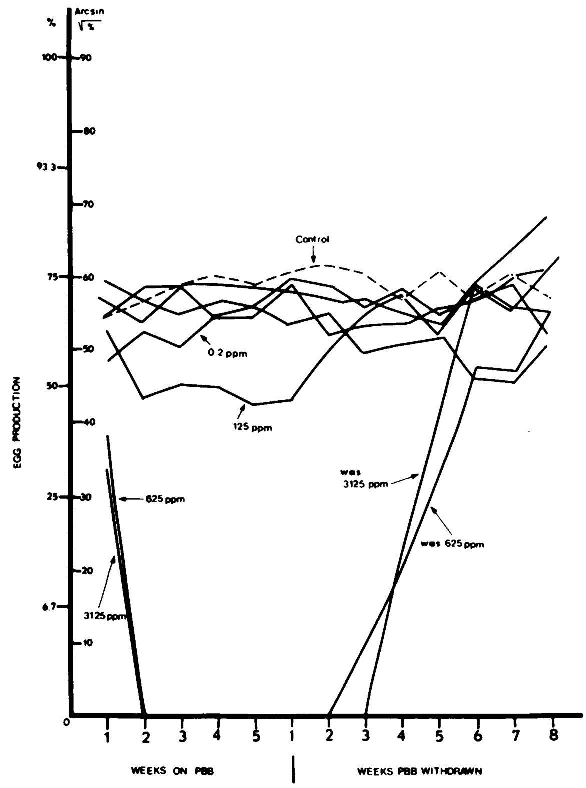

FIGURE 1. Effect of PBB in hen diets at levels of 0.2, 1, 5, 25, 125, 625, and 3125 ppm. Such treatments were given to 24 hens per group for 5 weeks, except for the level 3125 ppm which was given for 4 weeks. Treatments were followed by noncontaminated feed for 8 weeks.

declined from 66% to 48%, and returned to normal within 2 weeks after withdrawal of PBB diet. No other significant decline in egg production was detected for birds treated with levels lower than this. The data on the hens fed 0.2 ppm PBB were interpreted to be that their production was characteristic of that group and was not an effect from PBB, particularly because production averaged almost the same at about 65% for the weeks during and after feeding PBB. A clearer picture of the effect of PBB on egg production was obtained in the second experiment in which PBB levels ranged from 30 to 120 ppm (Fig. 2). Egg production declined significantly ($p < 0.05$) when PBB was in the diet at levels greater than 30 ppm.

Most interesting to note is that following withdrawal of PBB diets, egg production returned to normal within 2 weeks if the hens had been fed PBB at 120 ppm or less, within 3 to 4 weeks if formerly fed 125 ppm, and within 5 to 6 weeks if formerly fed 625 or 3125.

The minimum level of PBB to cause a decline in egg production was determined to be between 30 and 45 ppm.

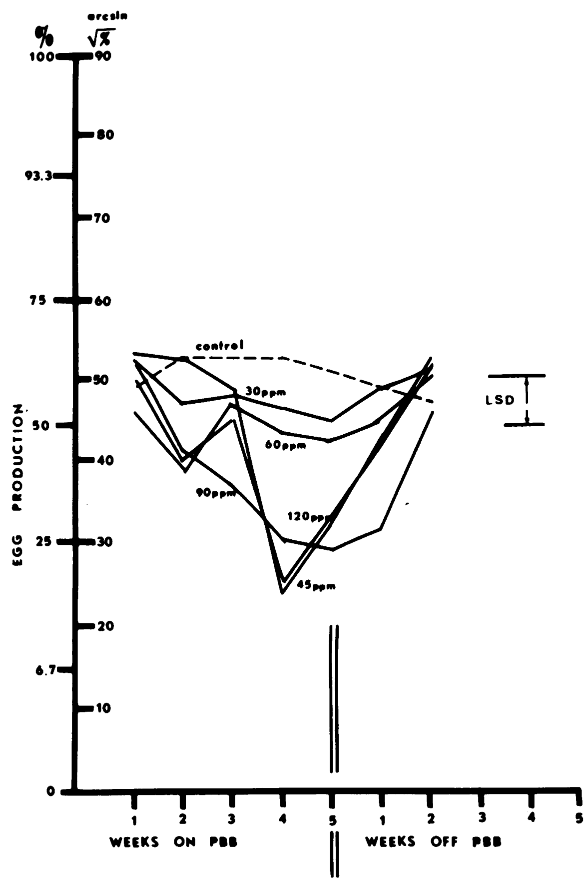

FIGURE 2. Effect of PBB on egg production of chickens given levels of 30, 45, 60, 90, and 120 ppm in their diet. The least significant difference (LSD) illustrated on the figure was calculated for a *p* value of 0.05.

Hatchability

No effect on hatchability occurred in experiment 1 when PBB was in the diet at 25 ppm or less (Fig. 3). Eggs obtained during the first week of the experiment from hens fed 125 ppm PBB hatched normally; but in subsequent hatches the values for hatchability were progressively lower reaching zero at the 5th week. A few eggs were obtained during the first week from hens given PBB in their diets at 625 or 3125 ppm. Already, an adverse effect on hatchability was detected.

Upon withdrawal of the PBB diets, hatchability of eggs from hens previously fed 125 ppm PBB recovered rapidly (Fig. 3). On the other hand, those eggs obtained from the hens previously fed 625 or 3125 ppm PBB exhibited a high embryo mortality, although egg production by this time was normal.

The data procurred from experiment 2 revealed that a borderline effect on hatchability was detected at 30 ppm and a definite effect at 45 ppm PBB in the

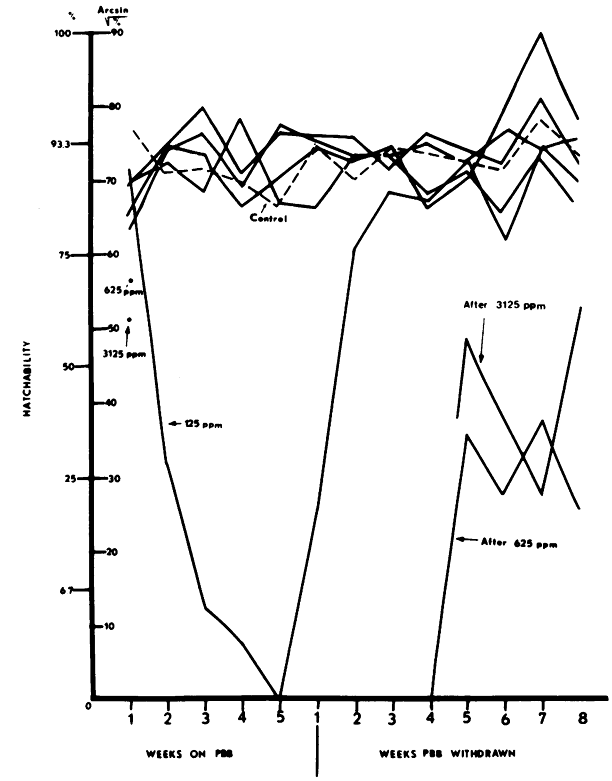

FIGURE 3. Effect of PBB on the hatchability of eggs from chickens given 0, 0.2, 1, 5, 25, 125, 625, or 3125 ppm in their diet.

diet (Fig. 4). Eggs did not hatch when collected from the fourth week on PBB through the first week off PBB when their source was the hens that had been fed 120 ppm levels of PBB, hatchability returned to the range of control values.

The minimum effective level of PBB for an effect on hatchability was established to be greater than 30 ppm but less than 45 ppm in the diet of laying chickens.

Offspring Mortality

Chicks that hatched from eggs of hens fed PBB were raised using a chick ration without PBB. Treatments applied to the hens in experiment 2 were PBB levels of 30 to 120 ppm for 5 weeks. Eggs require about 9 days for PBB to reach a plateau (based on the weekly analysis of the eggs from experiment 1) and require about 9 days following withdrawal of PBB for a change to occur in PBB concentrations. (Yolks are formed in about 9 days and PBB is lipotropic.) Thus, the offspring obtained from the hens treated with PBB during weeks 2 to 5, and the first week after withdrawal (−1) were evaluated for carryover through the egg. Table 2

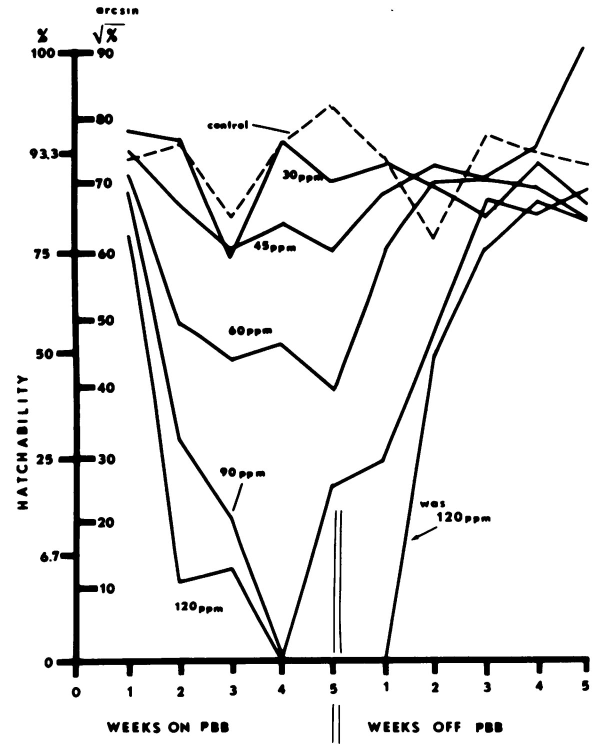

FIGURE 4. Effect of PBB on the hatchability of eggs from hens given 0, 30, 45, 60, 90, or 120 ppm in their diet for 5 weeks.

lists the percent mortality of chicks according to the treatment applied to the hens. Offspring mortality increased linearly with increasing levels of PBB in the hens' diets according to the equation $Y = -54.42 + 50.00X$, where X = log of ppm PBB in diet, and $Y = \arcsin \sqrt{\%}$ of the mortality. Allowing for 2σ as a difference between control and a treated group for a significant effect at $p = 0.05$, the estimate for a minimum detectable effect for PBB on offspring mortality was estimated at 42 ppm PBB in the hens' diets. An average (but nonsignificant) increase in chick mortality was detected with 30 ppm of PBB in the hens' diets, a significant ($p \leq 0.05$) increase at 45 ppm. Generally there was a tendency for chick mortality to be higher when orginally from eggs collected during weeks 2 to 4, than 5, and −1.

Tissue Residues

Muscle, liver, whole egg, and adipose samples obtained during the time PBB was fed had PBB residues which were linearly related to dietary PBB levels in a log–log relationship (Figs. 5, 6, and 7). Generally, the ratios of tissue PBB:diet PBB averaged 3:1 for adipose tissue, 1.5:1 for whole egg, 0.8:1 for liver, and 0.008:1 for muscle. The trend at which PBB accumulated in these tissues and whole egg with increasing levels of PBB in the diet, as based on slope values, were highest for muscle and egg (b = 1.015), intermediate for adipose (b = 0.923), and lowest for liver (b = 0.765). Controls had detectable PBB of 0.04, 0.08, 0.07, and 0.21 for muscle, liver, whole egg, and adipose tissues, respectively. This indicated that PBB dust from the contaminated diets was settling on the feed given to the control hens housed in the same room. At a time subsequent to the experiment, three hens housed in a different area of the campus were sacrificed and adipose tissues included in the samples submitted for analysis. Their value of 0.07 ppm represented a clean adipose tissue sample for comparison to samples from hens on the experiment.

The withdrawal curves for PBB from muscle, liver, whole egg, and adipose tissue are plotted in Figures 8, 9, 10, and 11, respectively. Those for muscle indicate a PBB half-life ($t_{½}$) of approximately 17 days, based on the average slopes for the former treatment levels of 5 to 625 ppm PBB in the diet and a withdrawal time of 56 days. When treatments had been 0.2 to 125 ppm PBB in the hens' diets, a biological half-life of 17 days was calculated also for whole eggs analyzed during the 56 days of withdrawal. The variability of the data on liver PBB levels following withdrawal made an estimated $t_{½}$ rather tenuous. A value was calculated based on the average slope for the lines depicting 1 to 125 ppm, and the 625 ppm line for days 28 to 56. That value for liver $t_{½}$ was estimated at 31 days, as based on the 56 days of withdrawal. Analysis of the data on adipose tissue (Fig. 11) revealed that PBB levels remained unchanged over the 56 days of withdrawal.

Table 2. Percent of mortality of offspring from hens fed PBB, experiment 2; offspring reared on feed without PBB.

PBB in hen diet, ppm	Mortality in offspring of hens fed PBB for varying periods, %					
	2 weeks	3 weeks	4 weeks	5 weeks	−1 week	Mean[a]
0	0	13	6	3	3	3.7 (± 4.8)
30	20	20	10	6	6	11.7 (± 6.3)
45	26	26	25	10	16	20.1 (± 5.5)
60	36	26	50	40	20	34.0 (± 7.1)
90	35	75	67	11	50	46.7 (± 16.1)
120	67	[b]	—	—	[b]	?

[a] Means are based on transformation of values to $\arcsin \sqrt{\%}$; mean (± std. dev.).
[b] Inadequate numbers.

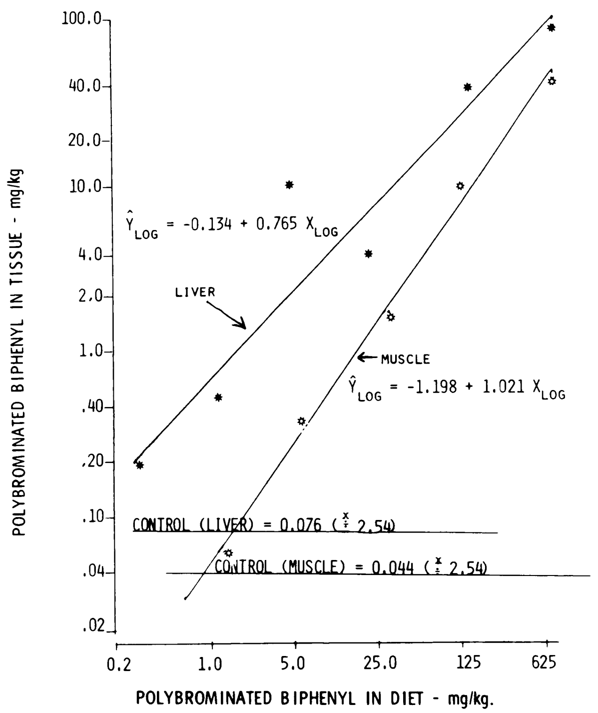

FIGURE 5. Relationship between PBB in the diet and the PBB detected in muscle and liver samples (mg/kg = ppm).

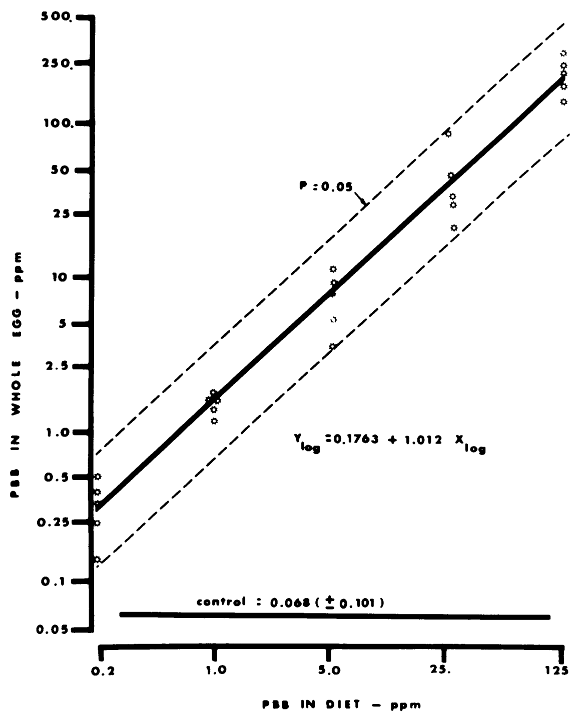

FIGURE 6. Relationship between PBB in the diet and PBB detected in whole egg. There were five pooled samples analyzed for each level of PBB in the diet. The dashed lines illustrate confidence limits for an individual statistic at $p = 0.05$.

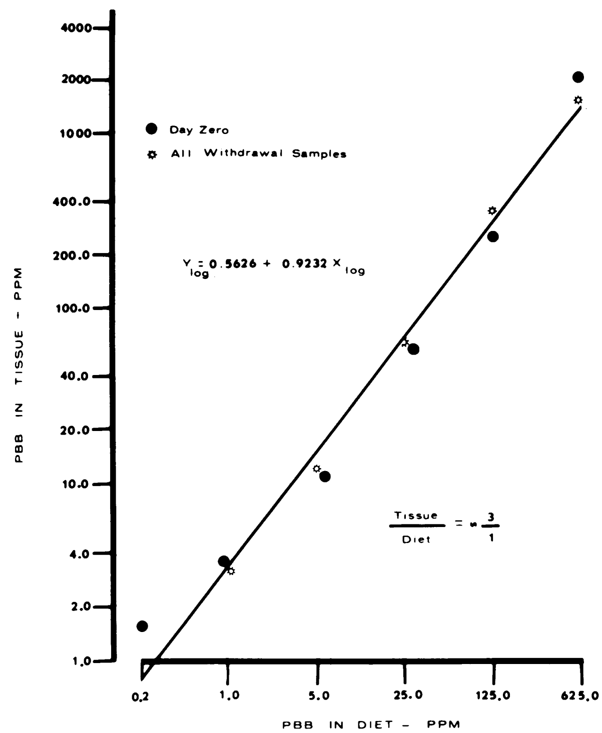

FIGURE 7. Relationship between PBB in the diet and the PBB detected in adipose tissue.

Discussion

The minimum level of PBB in the diet to produce a particular adverse effect on chickens and their progeny can be summarized as follows: feed intake, 125 ppm; egg production, 45 ppm; hatchability, 30 to 45 ppm; offspring mortality, 30 to 45 ppm.

Data from the USDA laboratory (*4*) indicated that 64 ppm PBB had no effect on feed intake, substantiating our data from experiment 2, and that 640 ppm caused a refusal of feed, substantiating our data from experiment 1. Babish et al. (*7*) noted that 100 ppm PBB in the diet to Japanese quail had no effect, whereas 500 or 1000 ppm produced inanition.

The USDA laboratory in two studies (*2*, *4*) reported that 20 ppm PBB produced an adverse effect on production and hatchability of eggs from chickens. This is a level slightly below the minimum effective level reported by us and is also in contrast to the data reported by Babish et al. (*7*). The latter reported that no such adverse effects were detected when Japanese quail were fed 10 to 20 ppm PBB in a semipurified type diet.

No adverse effects on egg weight or on quality of the egg's components occurred at any of the PBB levels on which eggs were produced, particularly at levels which reduced egg production. Similar reports have appeared from other studies on chickens

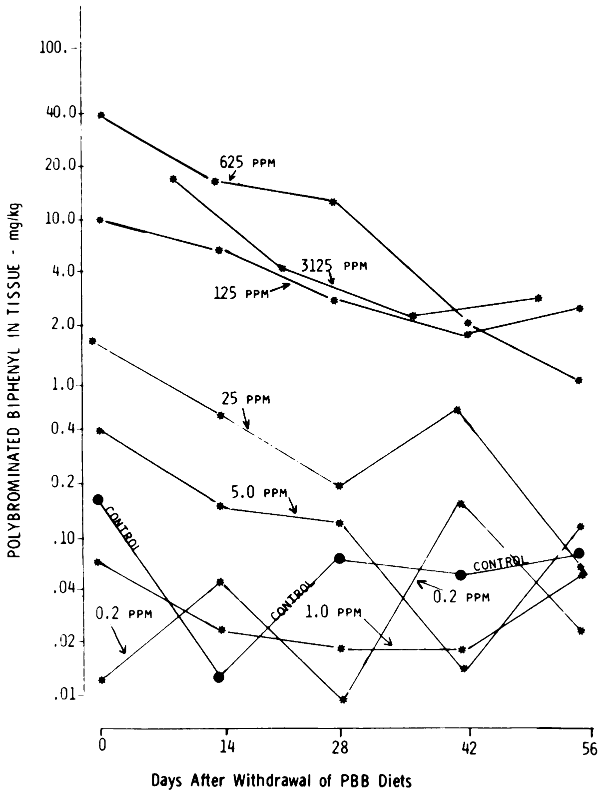

FIGURE 8. Levels of PBB in muscle samples from laying hens at the time they had been fed diets with PBB for 5 weeks (0 days), and at subsequent times after withdrawal of the PBB diets. Levels of PBB in control samples most likely occurred from dust of PBB diets settling on the feed of these hens kept in the same room.

(*4*) and Japanese quail (*7*).

The carryover of PBB in the egg was at a level approximately 1.5 times the dietary level when both are considered on an "as is" basis. As pointed out previously (*5*), Babish et al. (*7*) reported an egg:diet ratio of 1.3 when their data are converted from a dry weight to an "as is" basis. Dose-response curves for diet level of PBB versus PBB in whole egg allowed an estimate of 63 ppm (range 25–150 ppm) in egg at the estimated minimum effective level of 40 ppm for an adverse effect on hatch and offspring from PBB in the diet. On the estimate that a 59 g egg contents yields a chick of 45 g and that all of the PBB resides in the chick at hatching, then the body burden of PBB at hatch would be estimated at 3.72 mg/45 g chick or 82.6 mg/kg body weight. This would change markedly as the chick grew and would be reduced nearly 5-fold by day 21. Yet one has to recognize that this amount is producing a borderline toxicity.

Despite very high levels of PBB in the adipose tissue of hens during the entire withdrawal period, eggs laid by hens previously fed levels of PBB up to

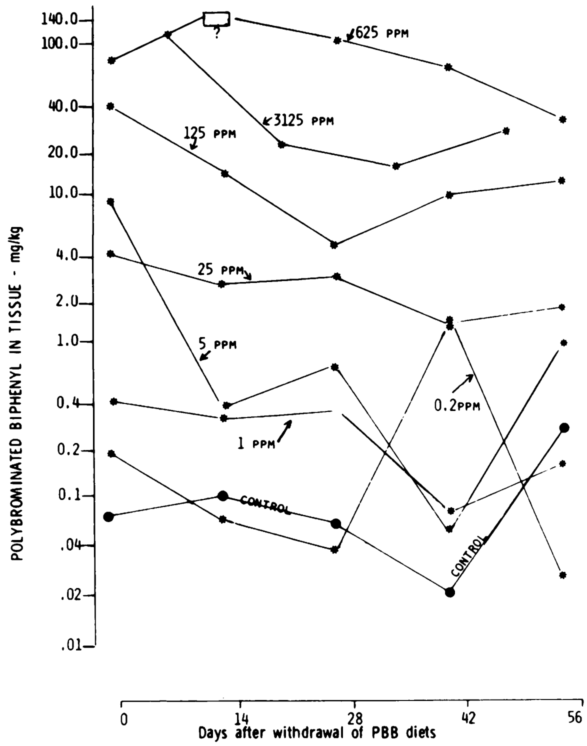

FIGURE 9. PBB levels in liver samples taken from hens at the time they had been fed PBB diets for 5 weeks (0 days) and at subsequent times after withdrawal of PBB diets. The value for day 14 off 625 ppm PBB was an estimated value based on the response curve for values obtained on days 28, 42, and 56 off 625 ppm PBB.

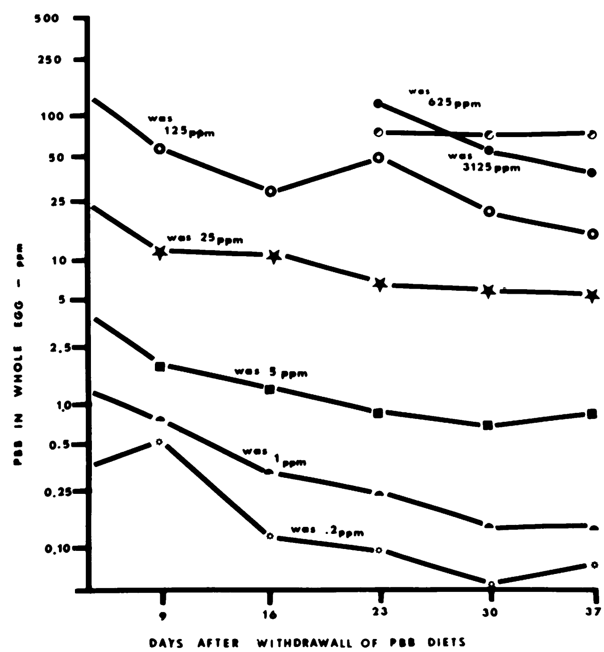

FIGURE 10. PBB levels in whole egg samples procured at the stated time that PBB diets had been withdrawn after being fed for 5 weeks.

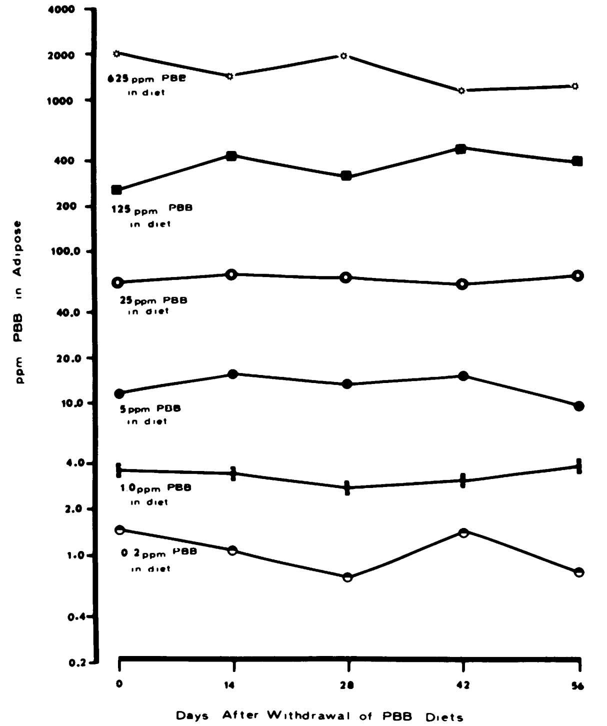

FIGURE 11. PBB in adipose tissue of laying hens at the time PBB diets were withdrawn after 5 weeks of feeding (0 days) and at subsequent times.

125 ppm showed a recovery to normal hatches and egg production within 2 to 4 weeks. PBB in the egg declined during this time but were shown to be present at substantial levels averaging 10 and 30 ppm in eggs from hens previously fed 25 and 125 ppm PBB in the diet. Therefore, the presence *per se* of PBB in eggs was not indicative that an embryotoxicity would ensue. Fries (*1*) showed that hepta-BB underwent a faster withdrawal from eggs than hexa-BB upon withdrawal of PBB from the diets. Although additional data are required before definite conclusions can be stated about particular PBB isomers and toxicity, one must recognize from the data just discussed, that such relationships must be considered. Body burdens of PBB can exist without any evidence of toxicity. Thus, PBB is similar to other poisons in that it too has a toxicity which is titratable down to a minimum.

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